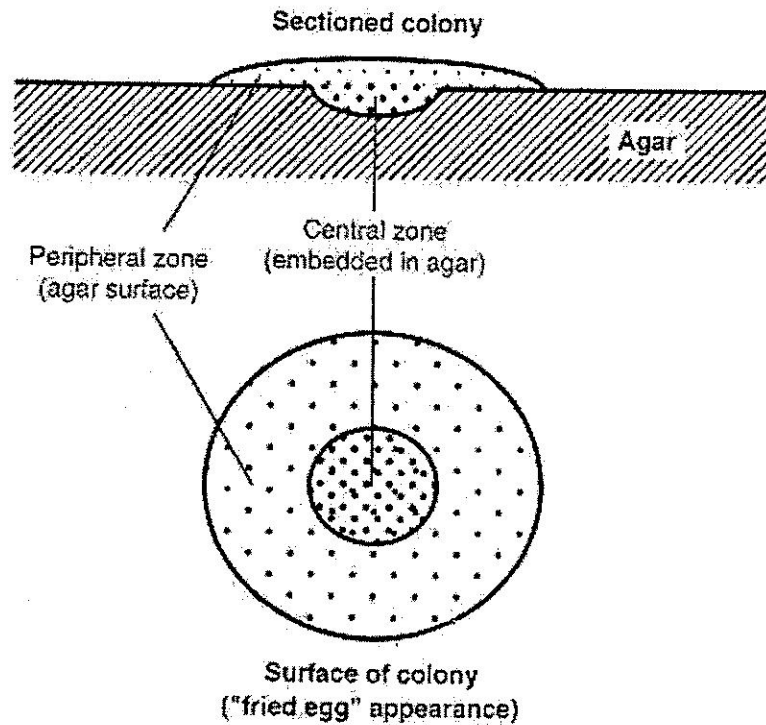


# Gram – ve Bacteria

## Class: Mollicutes ( Mycoplasmas )



أ.د/ جمال يونس

# **Phylum(Division): Firmicutes**

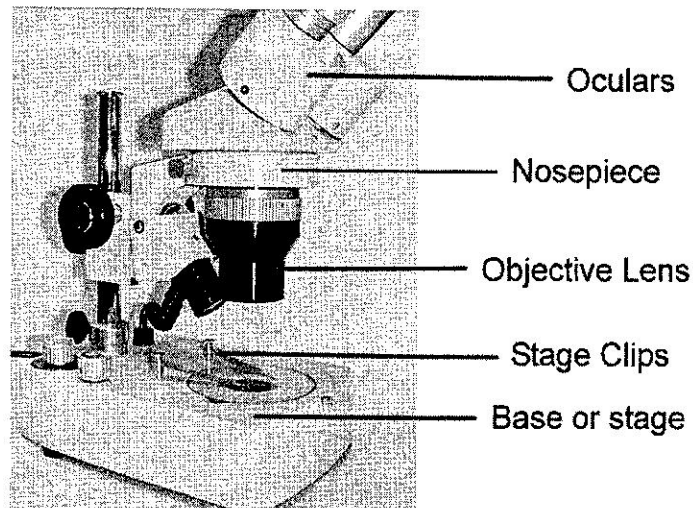
## **CLASS : MOLLICUTES**

### **Family: Mycoplasmataceae**

#### Recent Taxonomic classification of Mycoplasmas:

under phylum (division) Firmicutes due to low GC – content (18 – 40 mol %) small genome.

#### **Dissecting Microscope**



# Class: Mollicutes

- ♦ Lack cell wall and do not synthesize peptidoglycan.
- ♦ Mollicutes are bacteria which have small genomes, Lack a cell wall and have a low GC-Content (18 - 40 mol %).

## Classification:

order: Mycoplasmatales

Family: Mycoplasmataceae  
(Choleplasmataceae)

Genus I: Mycoplasma

Species:

a-avian mycoplasmas:

- ♦ M. gallisepticum
- ♦ M. meleagridis
- ♦ M. synoviae
- ♦ M. iowae

b-animal mycoplasmas:

- ♦ M. mycoides
- ♦ M. bovis
- ♦ M. bovis genitalium
- ♦ M. bovoculi

order: Acholeplasmatales

Family: Acholeplasmataceae  
(does not need cholesterol for its growth)

Genus: Acholeplasma

Genus II: Ureaplasma

(require urea for its growth)

## pathogenicity:

Species	disease
<b>(a) avian mycoplasmas:</b>	
♦ M. gallisepticum	avian mycoplasmosis ♦ <b>CRD</b> in chickens (with other bacteria mainly E. coli) ♦ Infectious sinusitis in turkeys.
♦ M. meleagridis	air-sacculitis and bursitis in turkeys
♦ M. synoviae	synovitis in chicken and turkeys
♦ M. iowae	air-sacculitis, stunting and Leg deformities.
<b>N.B:</b> These 4 species are <u>egg transmitted</u> .	
<b>(b) animal mycoplasmas:</b>	
1- M. mycoides	
♦ subsp. mycoides	<b>CBPP</b> (Contagious bovine pleuropneumonia) in cattle.
♦ subsp. Capri	<b>CCPP</b> (Contagious Caprine pleuropneumonia) in goats.
2- M. bovis	Mastitis and arthritis in cattle
3- M. bovis genitalium	Vaginitis, arthritis, mastitis and seminal vesiculitis in cattle
4- M. bovoculi	Keratoconjunctivitis in cattle
5- M. agalactiae	Contagious agalactia in sheep and goats
6- M. ovipneumoniae	pneumonia in sheep.

## general characters of Mycoplasmas :

- ① Gram -ve bacteria
- ② stain poorly with Gram's stain but they produce better results with Giemsa and Romanowsky stains.
- ③ IntraCellular bacteria
- ④ They are devoid of cell wall peptidoglycan  
→ So, they have not constant shape (pleomorphic).
- ⑤ The Cytoplasm is surrounded by plasma membrane which is a triple-layered and contain sterol (unit-membrane).
- ⑥ They are the smallest bacteria (0.1-0.3  $\mu\text{m}$  in diameter).
- ⑦ Non-motile
- ⑧ growth requirements:
  - 1-  $\text{O}_2$  requirement:
    - ◆ aerobic or facultative anaerobic
    - ◆ Some of them require 5%  $\text{CO}_2$  to grow (Capnophilic)
    - ◆ others are strict anaerobic (called anaeroplasm)
  - 2- opt. temp for growth → 35 - 37°C
  - 3- opt. pH → 7.2 - 7.8
  - 4- They need :
    - ◆ high conc. of Serum (10-15%), cholesterol or NAD Factor to grow.

- Inhibitors e.g.
  - penicillin (KILL G<sup>+</sup>ve bacteria)
  - thalium acetate (KILL G<sup>-</sup>ve)
  - amphotericin B (KILL fungi)

This is because mycoplasmas Lack cell wall  
→ so, they are resistant to penicillin that  
used for the inhibition of contaminants.

### 5. Media used:

- Mycoplasma agar and broth
- pplo media

→ produce Fried egg micro-colonies

- They are the smallest colonies (0.1-0.6 mm in diameter) → visible under dissecting or stereomicroscope or low power of ordinary microscope or hand lens.

- The micro-colonies are ch' by → an opaque granular center with a deep growth in the medium and a flat transparent zone of growth at periphery.

flat transparent zone of growth at periphery

opaque granular center

deep growth in the medium

## Laboratory diagnosis :

### ① Isolation :

#### ◆ O<sub>2</sub> requirement :

- aerobic or facultative anaerobic
- Some require 5% CO<sub>2</sub> to grow (Capnophilic)

◆ opt. temp. → 35 - 37 °C

◆ opt. pH → 7.2 - 7.8

#### ◆ Incubation time :

The growth is slow → so, incubation of cultures should be continued from 2-4 days to 14 days in a humid atmosphere before being regarded as -ve.

#### ◆ Specific media of mycoplasmas :

- Mycoplasma agar and broth
- heart infusion broth and agar
- ppLo media

#### → Contain inhibitors :

- penicillin (kill Gram +ve bacteria)
- Thallium acetate (kill Gram -ve bacteria)
- amphotericin-B (to kill fungi)

◆ obtaining a pure culture is done by culturing either on

- agar - broth - agar
- agar - agar

## ② Identification:

### a. Culture characters:

- on Liquid media → slight turbidity
- on Solid media → fried egg micro-colonies
- differentiation between mycoplasma micro-colonies and bacterial L-forms:

1. subculture on media without antibacterial substances (up to 5 subcultures) → Mycoplasma micro-colonies remain very small.

2. stain the micro-colonies with Dienes's stain →

→ Mycoplasma micro-colonies retain the stain indefinitely.

→ bacterial L-forms colonies → decolourize in about 15 min.

### b. Morphology:

of little value due to:

1. weak staining by various methods.
2. pleomorphism (Mycoplasma is an originally pleomorphic organism because they lack cell wall and do not synthesize peptidoglycan)



### c. Identification of the genus:

by digitonin test (sensitivity to digitonin)

Sensitive

(Inhibition of growth by digitonin)

Modified urease test

urease -ve

Mycoplasma  
(Colonies →  
0.1 - 0.6 mm in  
diameter)

Resistant

(growth)

↓  
Acholeplasma  
(Colonies →  
upto 3 mm in  
diameter)

urease +ve

ureaplasma  
(Colonies →  
0.01 - 0.05 mm in  
diameter "Tiny or  
T-mycoplasmas")

### d. Identification of species:

① Biochemical tests: such as

- ♦ glucose fermentation
- ♦ arginine hydrolysis
- ♦ phosphatase activity
- ♦ Reduction of tetrazolium

## ② Serological tests (Sero-diagnosis):

a. For mycoplasmal antigens (using known antisera)

1- FAT (direct or Indirect)

used Commonly With avian mycoplasmas

2- agar gel diffusion

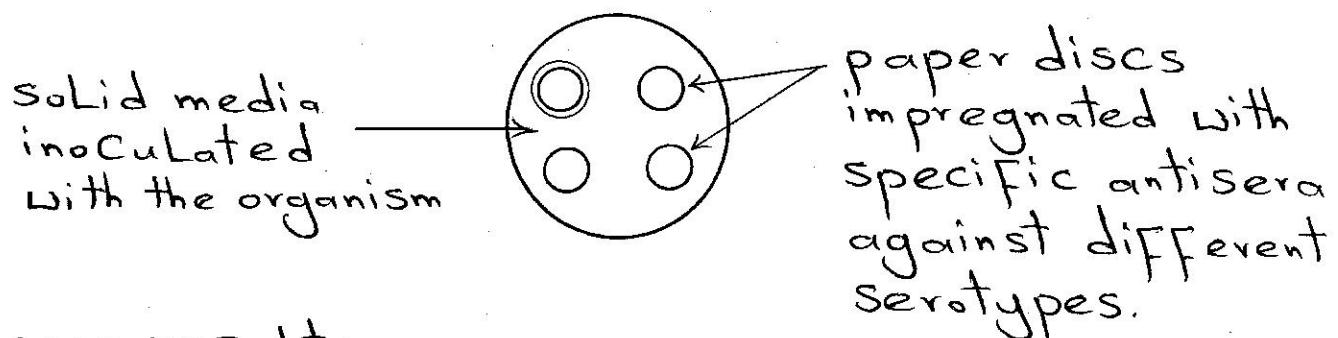
3- ELISA

4- CFT

5- growth inhibition test:

### growth inhibition test:

depend on the inhibition of growth of mycoplasma on solid media by the potent specific antisera.



+ve result:

Zone of growth inhibition around the disc

b. For mycoplasmal antibodies

(using known antigen)

1- plate (rapid) agglutination test

(Serum plate agglutination test, SpA)

→ using coloured stained killed Ag

→ For avian mycoplasmas

## 2 - Haemagglutination inhibition test (HI):

- For avian mycoplasmas
- more sensitive than SpA

3 - Indirect haemagglutination

4 - ELISA

5 - CFT

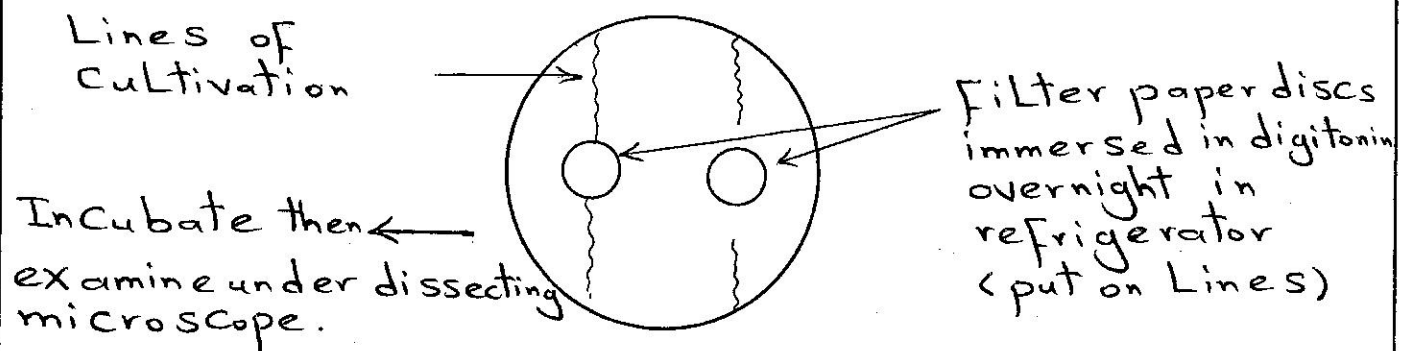
6 - agar gel diffusion test → For avian mycoplasma.

## ③ Molecular biological tests:

- PCR
- DNA probes

## digitonin sensitivity test:

→ used to differentiate between Mycoplasma and acholoplasma (non-pathogenic)



• IF no growth → Mycoplasma (sensitive to digitonin)

## Susceptibility to antibiotics:

- Mycoplasmas are resistant to antibiotics which act on cell wall such as penicillins.
- They are sensitive to antibiotics which inhibit protein synthesis such as tetracyclins and erythromycin.

## specific pathogen free (spf) programs:

to obtain poultry flocks free from the major avian mycoplasmas → by

- ① detection of infections and control of infected birds.
- ② periodic serological monitoring of the flocks to demonstrate continued freedom from infection.

## Genus: Ureaplasma

### U. urealyticum:

Cause non-gonococcal urethritis in man.

- Colonies → very very small (0.01-0.05 mm in diameter) → so, they are called T. mycoplasmas (Tiny mycoplasmas)
- require urea for its growth in addition to other requirements of Mycoplasma.

- pH  $\longrightarrow$  6.0
- Thallium acetate  $\longrightarrow$  not used in the medium because it is harmful to ureaplasma species.

### Modified urease test:

aim:

biochemical test used for differentiation between Mycoplasma and ureaplasma.

### Technique:

Mixture of equal parts of 10% urea soln. + 0.8% manganese chloride  $\longrightarrow$  apply directly onto 40 hrs old microColonies



+ve result

Immediate colour reaction goes from Light to dark brown and finally to black (due to deposition of manganese on the surface of Colonies).

+ve result  $\longrightarrow$  ureaplasma  
-ve result  $\longrightarrow$  Mycoplasma